APPLICATION FOR FEDERAL ASSISTANCE

Version 7/03

		2. DATE SUBMITTED 9/1/2006	Applicant Identifier
1. TYPE OF SUBMISSION: Application ☐ **Construction** ☑ **Non-Construction**	Pre-application ☐ **Construction** ☐ **Non-Construction**	**3. DATE RECEIVED BY STATE**	State Application Identifier
		4. DATE RECEIVED BY FEDERAL AGENCY	Federal Identifier

5. APPLICANT INFORMATION

Legal Name: University of Southern California	**Organizational Unit:** Department: Preventive Medicine
Organizational DUNS: 072933393	Division: Environmental Health
Address: Street: 2250 Alcazar Street, CSC 219	**Name and telephone number of person to be contacted on matters involving this application (give area code)** Prefix: Ms. / First Name: Sarah
City: Los Angeles	Middle Name J.
County: Los Angeles	Last Name Cusimano
State: CA / Zip Code 90033	Suffix:
Country: USA	Email: cusimano@usc.edu
6. EMPLOYER IDENTIFICATION NUMBER *(EIN):* 95-1642394	Phone Number (give area code) (323) 442-2396 / Fax Number (give area code) (323) 442-2835
8. TYPE OF APPLICATION: ☐ **New** ☑ **Continuation** ☐ **Revision** If Revision, enter appropriate letter(s) in box(es) (See back of form for description of letters.) ☐ ☐ Other (specify)	**7. TYPE OF APPLICANT:** (See back of form for Application Types) Other (specify)
	9. NAME OF FEDERAL AGENCY: Environmental Protection Agency
10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER: ☐☐-☐☐☐ TITLE (Name of Program):	**11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:** Children's Environmental Health Center
12. AREAS AFFECTED BY PROJECT *(Cities, Counties, States, etc.):* California	
13. PROPOSED PROJECT Start Date: 11/1/2006 / Ending Date: 10/31/2007	**14. CONGRESSIONAL DISTRICTS OF:** a. Applicant / b. Project

15. ESTIMATED FUNDING:

a. Federal	$ 1,433,828.00
b. Applicant	$ .00
c. State	$ .00
d. Local	$ .00
e. Other	$ .00
f. Program Income	$ .00
g. TOTAL	$ 1,433,828.00

16. IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. Yes. ☐ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON DATE:

b. No. ☐ PROGRAM IS NOT COVERED BY E. O. 12372

☐ OR PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. IS THE APPLICANT DELINQUENT ON ANY FEDERAL DEBT?

☐ Yes If "Yes" attach an explanation. ☑ No

18. TO THE BEST OF MY KNOWLEDGE AND BELIEF, ALL DATA IN THIS APPLICATION/PREAPPLICATION ARE TRUE AND CORRECT. THE DOCUMENT HAS BEEN DULY AUTHORIZED BY THE GOVERNING BODY OF THE APPLICANT AND THE APPLICANT WILL COMPLY WITH THE ATTACHED ASSURANCES IF THE ASSISTANCE IS AWARDED.

a. Authorized Representative

Prefix Ms.	First Name Sarah	Middle Name J.
Last Name Cusimano		Suffix
b. Title Associate Director		c. Telephone Number (give area code) (323) 442-2396
d. Signature of Authorized Representative (b) (6) [redacted]		e. Date Signed 8/31/06

Previous E[illegible]
Authorized for Local Reproduction

Standard Form 424 (Rev.9-2003)
Prescribed by OMB Circular A-102

Principal Investigator/Program Director (Last, first, middle): Gilliland, Frank D.

Project 2 - Subcontract to UCLA

DETAILED BUDGET FOR NEXT BUDGET PERIOD -- DIRECT COSTS ONLY	FROM 11/01/06	THROUGH 10/31/07	GRANT NUMBER 5P01ES 009581-09

PERSONNEL (Applicant organization only)		Months Devoted to Project			DOLLAR AMOUNT REQUESTED (omit cents)		
NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Sum. Mnths	SALARY REQUESTED	FRINGE BENEFITS	TOTALS
Diaz-Sanchez, David	Principal Investigator	12.00	35.00		(b) (6)		
Wang, JunXiang	PGR	12.00	100.00				
Casillas, Adrian	Co-Investigator	12.00	21.00				
		0.00			$0	$0	$0
		0.00			$0	$0	$0
		0.00			$0	$0	$0
		0.00			$0	$0	$0
		0.00			$0	$0	$0
SUBTOTALS →					$102,697	$20,254	$122,951

CONSULTANT COSTS

Statistical consultation from the biostat Service Core of the SCEHSC $2,864

EQUIPMENT *(Itemize)*

SUPPLIES *(Itemize by category)*

Plasticware, tubes, disposable pipettes, etc. Immunoassay materials, Molecular assay materials,immunohostochemical reagents, cell culture reagents $14,720

TRAVEL

Attend annual meeting $1,236

PATIENT CARE COSTS — INPATIENT

PATIENT CARE COSTS — OUTPATIENT

ALTERATIONS AND RENOVATIONS *(Itemize by category)*

OTHER EXPENSES *(Itemize by category)*

SUBTOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD		**$ $141,771**
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS (LAREI)	$58,988
	FACILITIES AND ADMINISTRATION COSTS	$7,904
TOTAL DIRECT COSTS FOR NEXT PROJECT PERIOD *(Item 8a, Face Page)*		**$ $208,663**

Principal Investigator/Program Director (Last, First, Middle): Gilliland, Frank D.

BUDGET JUSTIFICATION	GRANT NUMBER
	5P01ES009581-09

Provide a detailed budget justification for those line items and amounts that represent a significant change from that previously recommended. Use continuation pages if necessary.

No Significant Changes

CURRENT BUDGET PERIOD	FROM	THROUGH
	11/1/2005	10/31/2006

Explain any estimated unobligated balance (including prior year carryover) that is greater than 25% of the current year's total budget.

We do not anticipate an excess of 25% to be carried over into the next year.

PROGRESS REPORT SUMMARY	GRANT NUMBER 5 P01 ES009581-09	
	PERIOD COVERED BY THIS REPORT	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR Frank D. Gilliland, MD, PhD	FROM 11/1/05	THROUGH 10/31/06
APPLICANT ORGANIZATION University of Southern California		
TITLE OF PROJECT (Repeat title shown in Item 1 on first page) Children's Environmental Health Center		

A. Human Subjects (Complete Item 6 on the Face Page)		
Involvement of Human Subjects	☒ No Change Since Previous Submission	☐ Change
B. Vertebrate Animals (Complete Item 7 on the Face Page)		
Use of Vertebrate Animals	☒ No Change Since Previous Submission	☐ Change
C. Select Agent Research	☒ No Change Since Previous Submission	☐ Change
D. Multiple PI Leadership Plan	☒ No Change Since Previous Submission	☐ Change

SEE PHS 2590 INSTRUCTIONS.

WOMEN AND MINORITY INCLUSION: See PHS 398 Instructions. Use Inclusion Enrollment Report Format Page and, if necessary, Targeted/Planned Enrollment Format Page.

Project Number 2: Pollution-Enhanced Allergic Inflammation and Phase II Enzymes

A. Specific Aims

There has been no change in the specific aims of this study, they are to study the role of Phase II enzymes in regulating responses to pollutants in: children's upper airways (Aim #1); the lower airways of healthy and asthmatic individuals (Aim #2) and in mechanistic animal and cellular models of allergic inflammation (Aim #3).

B. Studies and Results

Aim #1: **We will test the hypothesis that Phase II enzyme expression in the upper airways are induced by oxidant pollutants and differ between children and adults.**

Last year we reported on phase II enzyme expression following nasal challenge with Diesel Exhaust Particles (DEP) in 10 adult subjects. In the last year we have expanded these studies and have completed similar challenges on an additional 10 adult subjects and 20 children. This was a single-blind randomized cross-over exposure study to test the expression of these enzymes in response to nasal challenge with four different DEP doses - 0 (control), 30, 100 or 300 □g. Subjects performed nasal lavage sampling immediately before and 24 hours after each DEP challenge. DEP was administered by nasal spray into one nare via an atomizer. Each subject was randomized to one of 24 possible sequences of 4 DEP exposures. A four week wash-out period was observed between each DEP exposure. In adults, DEP enhanced production of IL-8, TNF-□ and GM-CSF in a dose-dependent fashion. Higher DEP challenge dose concentrations were associated with increased levels of chemokines as measured by ELISA. (see Fig1) In adults, DEP enhanced production of IL-8, TNF-□ and GM-CSF in a dose-dependent fashion. Higher DEP challenge dose concentrations were associated with increased levels of chemokines as measured by ELISA.

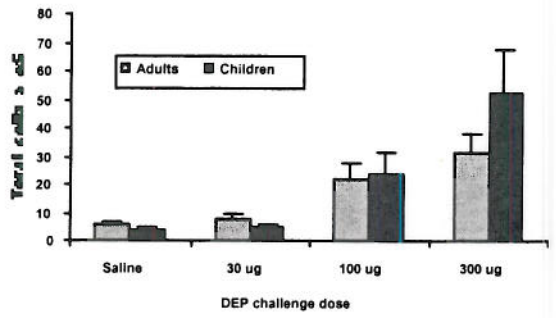

Fig 1 Cellular response to DEP in adults vs. children

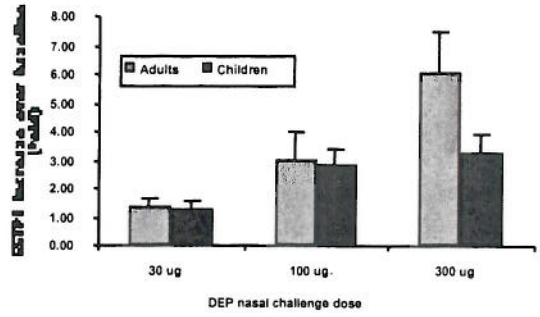

Fig 2 GSTP1 response to DEP in Adults vs. Children

The degree of cellular infiltration correlated with the dose of nasally administered DEP challenge. Higher concentrations of DEP elicited a larger number of total cells recovered from nasal lavage fluid obtained 24 hours after DEP exposure. As we have previously reported, we have developed real-time quantitative PCR (RT-PCR) to measure gene expression of sentinel Phase II enzymes (GSTM1, GSTP1, NQO1 and HO-1) from cells recovered from nasal washes. RNA was extracted from cells recovered from nasal lavages performed 24 hours after DEP challenge. In adults, DEP induced expression of GSTP1 in a dose-dependent fashion. With increasing concentrations of DEP challenge, there were higher levels of GSTP1 relative gene expression (Fig 2). In these adults, cellular infiltration was inversely correlated with GSTP1 enzyme gene expression. Increased levels of GSTP1 were associated with lower cell count numbers. In children, the degree of cellular infiltration also correlated with dose of nasally administered DEP challenge. Higher concentrations of DEP elicited a larger number of total cells recovered from nasal lavage fluid obtained 24 hours after DEP exposure. However, as compared to adults, this effect in children was more robust at the DEP challenge of 300 µg (see Fig 1). In these children, DEP also induced expression of GSTP1. At DEP concentrations of 30 and 100 µg, there were higher levels of GSTP1 relative gene expression. However, *GSTP1 relative gene expression for the DEP nasal challenge of 300 µg was decreased in children as compared to adults.* Similar results are seen with the other 3 Phase II enzymes measured.

Aim #2: **We will test the hypothesis that Phase II enzyme expression in the *lower* airways are induced by oxidant pollutants and differ between asthmatic and non-asthmatic subjects.**
In the past year we have exposed 10 subjects (5 asthmatic and 5 non-asthmatic) to diesel exhaust to study the effect of Phase II expression on lower airway responses. To date we have thus performed exposures on 10 healthy and 15 asthmatic subjects. We have refined our exposure system and can reproducibly produce a diesel particulate exposure level that is within 8% of the target level every time. These diesel particles are chemically and physically identical to diesel particles encountered in ambient air. Diesel exhaust resulted in increased expression of all four, phase II enzymes tested in cells recovered from sputum induced 24 hours after exposure. This increase was observed in both healthy and asthmatic subjects. There was wide heterogeneity in responses to diesel exhaust between subjects. While no enhanced inflammation was observed in any of the healthy subjects, four out of the fifteen asthmatic subjects demonstrated a robust inflammatory response to diesel exhaust but not filtered air. Phase II enzyme expression was measured in cells recovered from induced sputum performed 24 h after exposure. For all subjects measured to date we could observe an increase in phase II enzyme expression. Again there was wide heterogeneity in responses between subjects. This heterogeneity makes meaningful statistical analysis between asthmatic and healthy subjects premature.

Aim #3: **We will determine the role of Phase II enzymes in regulating the adjuvant effects of particulate pollutants.**

We have previously reported that treatment with sulforaphane (a phase II enzyme inducer) can prevent the production of pro-inflammatory cytokines in respiratory epithelial cells *in vitro* and IgE production from PBMCs. We have now explored this mechanism. We confirmed that DEPs and its extracts (DEPX) can act directly on B lymphocytes and showed that DEPX could enhance the IgH germline-transcription both in a B cell line and PBMC. DEPX increased NQO1 mRNA gene expression in both peripheral blood lymphocytes and the B lymphocyte cell line DG75 in a dose-dependent manner. NQO1 protein induction by DEPX in DG75 cells was confirmed by Western blot. A reporter gene assay showed that DEP could induce activity of the anti-oxidant response element (ARE) located in the NQO1 gene promoter. Induction of both NQO1 mRNA and protein expression could be blocked by co-culture with an anti-oxidant and partly repressed by inhibitors of PI3-K and p38 mitogen-activated protein kinase, but not by inhibitors of mitogen-activated protein kinase/extracellular signal-regulated kinase kinase (Mek/ERK) or protein kinase C (PKC).

C. Significance

The principal findings of our results is the discovery that children have enhanced inflammatory responses to the model pollutant DEP and that this seems to be related to their reduced capacity to make a cytoprotective Phase II enzymes response. Our in vitro data supports the idea that DEP suggest that phase II enzyme expression through activation of the ARE. Our studies illuminate why there may be increased susceptibility of certain vulnerable individuals and populations (such as children) to oxidant pollutants and suggest that increasing the body's Phase II responses either by therapeutic or dietary means may counteract this effect.

D. Plans

In the next year we intend to continue recruitment of adults and children for Aims #1 and #2 and use our invitro system to examine the role of individual Phase II enzymes.

E. Publications

1. Diaz-Sanchez, D., Riedl, M. Diesel effects on human health: a question of stress? Am J Physiol Lung Cell Mol Physiol. 289:L722-32005.
2. Diaz-Sanchez, D., Rumold, R., Gong Jr. H. Challenge with Environmental Tobacco Smoke Exacerbates Allergic Airway Disease in Humans. *J. Allergy Clin. Immunol.* In press
3. Wan, J., Diaz-Sanchez D. Phase II enzymes induction blocks the enhanced IgE production in B cells by diesel exhaust particles. *J. Immunol (in press).*
4. Ritz, S.A., Diaz-Sanchez D. Sulforaphane, a phase II enzyme inducer, Inhibits Cytokine Production by Airway Epithelial Cells Stimulated with Diesel Extract. (submitted).
5. Gilliland, F.D., Li, Y.-F., Gong Jr. H., Diaz-Sanchez, D. Glutathione-S-Transferase *M1* and *P1* Prevent Aggravation of Allergic Responses by Second-hand Smoke. *(submitted)*

Principal Investigator/Program Director (Last, First, Middle): Gilliland, Frank D.

Inclusion Enrollment Report

This report format should NOT be used for data collection from study participants.

Study Title: Children's Environmental Health Center – Project 2: Pollution-Enhanced Allergic Inflammation and Phase II Enzymes

Total Enrollment: 65 **Protocol Number:**

Grant Number: 5 P01 ES009581-09

PART A. TOTAL ENROLLMENT REPORT: Number of Subjects Enrolled to Date (Cumulative) by Ethnicity and Race

Ethnic Category	Sex/Gender			
	Females	Males	Unknown or Not Reported	Total
Hispanic or Latino	12	15		27 **
Not Hispanic or Latino	21	16		37
Unknown (individuals not reporting ethnicity)		1		65
Ethnic Category: Total of All Subjects*	33	32		*
Racial Categories				
American Indian/Alaska Native				
Asian	12	5		17
Native Hawaiian or Other Pacific Islander				
Black or African American		1		1
White	6	14		20
More Than One Race	15	11		26
Unknown or Not Reported				1
Racial Categories: Total of All Subjects*	33	32		65 *

PART B. HISPANIC ENROLLMENT REPORT: Number of Hispanics or Latinos Enrolled to Date (Cumulative)

Racial Categories	Females	Males	Unknown or Not Reported	Total
American Indian or Alaska Native				
Asian				
Native Hawaiian or Other Pacific Islander				
Black or African American				
White				
More Than One Race	8	8		16
Unknown or Not Reported	4	7		11
Racial Categories: Total of Hispanics or Latinos**	12	15		27 **

* These totals must agree.
** These totals must agree.